QIACUBE EXTRACTIONS

A. SCOPE

The QIAcube automates the DNA extraction process for biological evidence containing blood and/ or epithelial cells using the QIAamp Mini kits. The QIAcube controls integrated components including a centrifuge, heated shaker, pipetting system, and robotic gripper.

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure.

B.2 Each new QIAamp DNA Mini Kit lot must undergo quality control testing prior to extracting casework samples.

Biological material with known results along with a reagent control will be extracted using all the components of the kit undergoing quality control testing. The extracted material will be carried though the entire DNA analysis process. The results obtained from the known extracted sample must be as expected and good quality, as described in the GlobalFiler (DOC ID 12628) interpretation guidelines, for the kit to pass quality control testing. The quality control data will be placed into the critical reagent binder.

B.3 An analyst that dilutes the concentrated Buffers AW1 and AW2 prior to their initial use will be watched by a second analyst to confirm correct preparation. Both analysts will initial the bottle. In addition, the lot number and expiration date of the added ethanol will be recorded on the bottle.

B.4 See DOC ID 1835 to determine reagent expiration dates.

B.5 At least two reagent controls must be extracted along with a set of questioned samples.

B.6 Do not use spray bottles to spray cleaner onto surfaces of the QIAcube workstation.

B.7 Do not use alcohol or alcohol-based solutions to clean the QIAcube door. Clean the QIAcube door with deionized water.

B.8 Do not submerge buffer bottles in 70% alcohol as the blue ring is not ethanol resistant.

B.9 To clean touch screen, moisten a kimwipe with water or ethanol and carefully wipe display. Wipe dry with a kimwipe.

B.10 Do not use bleach, solvents, or any reagents containing acids, alkalis, or abrasives to clean the QIAcube and its accessories; instead use DNA Exitus Plus.

B.11 Empty the waste drawer and decontaminate the QIAcube with DNA Exitus Plus after each use.

B.12 When consuming a sample and the corresponding extract, you must keep the post extraction substrate (refer to the DNA Quality Manual DOC ID 1833 for details on evidence consumption and retention).

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 The sample preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with water or ethanol.

C.3 All appropriate SDS sheets must be read prior to performing this procedure.

C.4 Treat all biological specimens as potentially infectious.

C.5 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 QIAamp DNA Mini Kit

D.1.1 Buffer ATL
D.1.2 Buffer AL
D.1.3 Proteinase K
D.1.4 Buffer AW1

Before using for the first time, add 125 mL ethanol (Absolute) to 95 mL AW1 concentrate.

D.1.5 Buffer AW2

Before using for the first time, add 160 mL ethanol (Absolute) to 66 mL AW2 concentrate.

D.1.6 Buffer AE

D.2 Absolute Ethanol (200 proof)

D.3 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.4 70% ethanol (Decontamination)

D.5 DNA Exitus Plus (Decontamination of QIAcube)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 QIAcube
E.1.2 Scissors/Forceps
E.1.3 Microcentrifuge
E.1.4 Heat block
E.1.5 Pipettes
E.1.6 Vortexer

E.2 Supplies

E.2.1 Kimwipes
E.2.2 QIAcube sample tubes (2 mL, Qiagen P/N 990381)
E.2.3 Spin baskets
E.2.4 QIAcube Filter Tips, sterile aerosol-resistant
E.2.5 Racks
E.2.6 QIAamp mini rotor adapters (Part of Qiagen kit)
E.2.7 Spin columns (Part of Qiagen kit)
E.2.8 QIAcube elution tubes (Part of Qiagen kit)
E.2.9 Rotor adapter tray (Part of Qiagen kit)
E.2.10 Disposable gloves
E.2.11 Mask
E.2.12 Lab coat
E.2.13 Eye protection (e.g. safety glasses, face shield)
E.2.14 DNA Exitus Plus
E.2.15 Extraction sheet

F. PROCEDURE

Note: The QIAcube can process a maximum of twelve samples at a time per machine. It cannot process one or eleven samples.

F.1 Cut the sample and place into a labeled round bottomed 2.0 mL locking microcentrifuge tube provided by Qiagen.

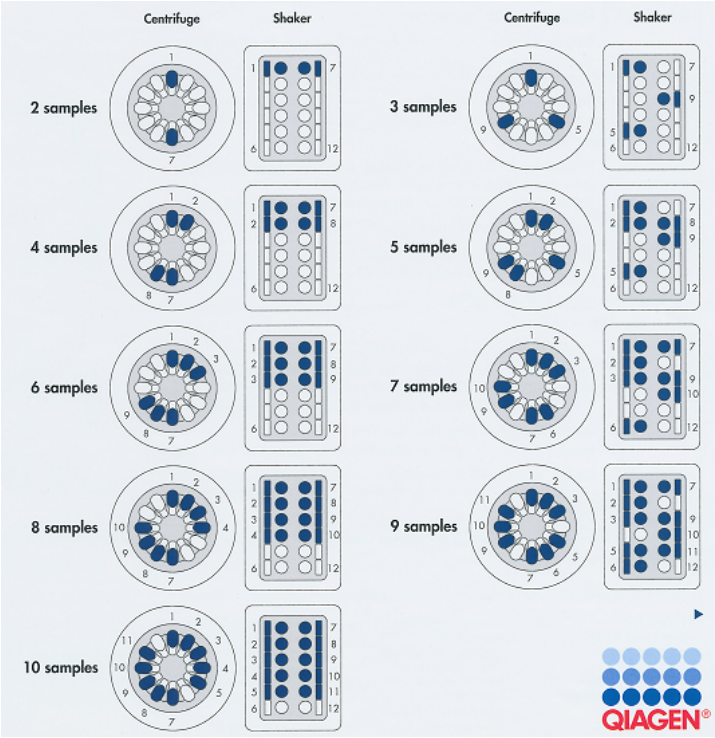
F.2 Place an opened 1.5 mL microcentrifuge tube with Proteinase K in position A on the QIAcube deck. Load a tray of light grey tips (1000 µL) and a tray of blue tips (200 µL) in the positions to the right of position A. **Make sure that the tips contain no pieces of plastic that may have chipped off of the plastic holder during shipment**.

F.3 Fill the provided reagent bottles with their respective reagents to just under the fill line. Filling the bottles above the fill line will elicit an error message.

F.4 Add the reagent bottles to their respective positions in the reagent bottle rack as seen in the diagram below. If reagent bottles have sufficient reagent and are already present on the deck, remove the lids and check that each reagent is in the correct location.

ATL	AL
EtOH (200 proof)	AW1
AW2	AE

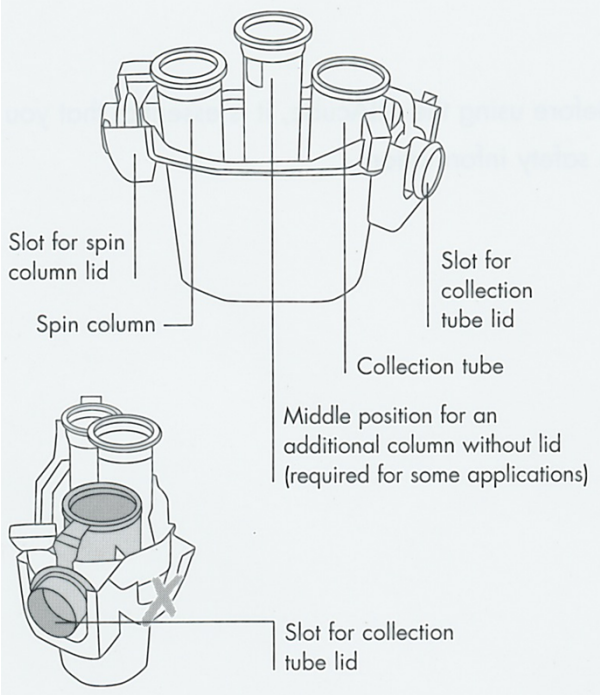
F.5 Load samples into the shaker according to the diagram below. Ensure all 2.0 mL tube caps are locked into position.



F.6 Close the lid of the QIAcube and turn the machine on (if it is not already on). To select the lysis protocol, select "DNA", "QIAamp DNA Blood Mini", "Buccal swab", and "Buccal swab lysis 60 min". Press start and follow the prompts the QIAcube will give. After the user has selected "yes" to all questions, the robot will begin the lysis protocol. Alternatively, select the lysis protocol using the shortcut displayed on the touch screen.

F.7 After the lysis protocol is complete, centrifuge the tubes. Using a pipette tip or sterile forceps, remove the evidence and place it in a spin basket. Place the spin basket into the original tube and centrifuge the tube for 5 minutes at maximum speed. When appropriate, discard / keep the substrate. If necessary, spin the tube again to pull down any liquid on the sides of the tube and then place the tube with lysate back into its original position on the QIAcube shaker.

F.8 Load the centrifuge with the assembled rotor adapters equipped with a QIAamp spin column and 1.5 mL collection tube provided by Qiagen. Refer to the diagram below. Assemble one rotor adapter per sample. Refer to the diagram in step F.5 for centrifuge loading instructions.



F.9 Close the lid of the QIAcube. To select the elution protocol, select "DNA", "QIAamp DNA Blood Mini", "Buccal swab lysate", and "Edit Elution". Alternatively, select the lysate protocol shortcut displayed on the touch screen. Press start and follow the prompts seen on the QIAcube touchscreen. After the user has selected "yes" to all prompts the robot will begin the elution protocol.

Note: The elution volume can be adjusted to elute in 50-100 uL. To adjust the elution volume press "Edit", then using the arrows, highlight the "elution volume", and then press "Select". Change the elution volume by pressing "+" or "-".

F.10 After the elution protocol is completed, remove the rotor adapters from the centrifuge. Remove and discard the QIAamp spin column from the 1.5 mL tubes. Cap and remove the 1.5 mL tubes containing the extracted material. Discard the rotor adapters.

F.11 Cap reagent bottles and Proteinase K. Empty and clean the tip waste drawer with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner. Wipe down the deck of the QIAcube and deck items with DNA Exitus Plus; this can be followed with a wipe down using 70% ethanol or deionized water. Clean the shaker rack with DNA Exitus Plus followed by a rinse with deionized water or 70% ethanol; bleach-based solutions must not be used on the shaker rack.

F.12 Save all data files associated with a QIAcube extraction on the USB stick provided with the QIAcube. These files should then be transferred from the USB stick to each analyst's

casework folder on the "I" drive. ONLY use the supplied QIAcube USB stick on the QIAcube to save all associated data files. To save the data files do the following: in the main menu press "Tools", "Data exchange", "Select", "Save all files", then "OK". These files must be saved on the same day of use to prevent loss of data; the QIAcubes can only retain nine report files at a time.

F.13 Quantitate (DOC ID's 1784 and 1785) the DNA and concentrate samples (DOC ID 1780) as necessary. Alternatively, samples may be concentrated prior to quantitation. Store sample extracts in the refrigerator when not in use. Sample extracts may be frozen for long-term storage.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 QIAcube User Manual, Qiagen, 6/2008.